UPTAKE OF ORGANIC MATERIAL BY AQUATIC INVERTEBRATES. IV. THE INFLUENCE OF SALINITY ON THE UPTAKE OF AMINO ACIDS BY THE BRITTLE STAR, OPHIACTIS ARENOSA [1]

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The ability to remove amino acids and other small organic compounds from dilute solution is widespread among marine invertebrates. Stephens and Schinske (1961) reported examples from ten different phyla. This capacity has been studied in additional forms and has provided material for a series of reports (Stephens, 1962, 1963, 1964; Stephens *et al.*, 1965; Virkar, 1963). It has been our experience that any soft-bodied marine invertebrate exposed to an amino acid such as glycine or phenylalanine at concentrations ranging between 10^{-5} and 10^{-6} moles per liter shows the capacity to remove it from solution quite rapidly. The fresh-water forms we have examined remove amino acids from solution very much more slowly, so slowly that we have not demonstrated the occurrence of the process unambiguously.

The relation between external salinity and the uptake of amino acids has proved to be of interest. Stephens (1964) showed that uptake of glycine in euryhaline nereid polychaetes occurred only at moderate to high salinities. At lower salinities, uptake stopped almost entirely. The salinity at which uptake ceased was closely correlated with that at which osmoregulation and chloride regulation began. The data did not permit firm conclusions about rates of uptake at intermediate salinities since they were acquired before recognizing the considerable capacity for adaptation in the system.

A related matter of interest is the regulation of the "free amino acid pool" in marine invertebrates in response to changes in salinity. The tissues and body fluids of most marine invertebrates are in osmotic equilibrium with their environment. Numerous workers have reported large amounts of non-protein nitrogenous substances in the tissues, of which amino acids are the most abundant (see Awapara, 1962; Kittredge *et al.*, 1962). This pool of amino acids is sufficiently concentrated to represent a major fraction of the osmotic concentration of the tissues. It has been shown that as salinity is decreased, the size of the pool decreases. Florkin (1962) has suggested that this behavior represents an osmoregulatory response in the sense that decreasing the size of the free amino acid pool spares larger fluctuations in other cellular constituents. This position is supported by a large number of observations carried out by Florkin and coworkers (reviewed in Florkin, 1962), as well as observations by Potts (1958), Shaw (1958), and Lange (1963, 1964). Virkar (1963, 1965) has studied the response of tissues of the sipunculid *Golfingia* to small changes in salinity. The change in free amino acids in the body wall which is produced by lowering the concentration of the ambient medium by 10% is

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large enough to account completely for the implied change in intracellular osmotic concentration.

The preceding paragraphs set a context in which several interesting questions can be asked. What is the source of free amino acids which form such a surprisingly concentrated pool in many marine organisms? Awapara (1962) argues it is not dietary, on the ground that there are differences in animals found in comparable habitats, but he does not make positive suggestions. By what means is the free amino acid pool in the tissues decreased in response to lowered salinity? We have no information whatever on this point. Does the free amino acid pool contribute significantly to the energy metabolism of the organism? What is the turnover rate of individual constituent acids in the pool?

The earlier work in our laboratory to which we have alluded is based on supplying uniformly labelled compounds to marine invertebrates in very dilute solution in the ambient medium. This provides a technique for labelling specific constituents of the free amino acid pool at will. The work to be reported uses this technique to provide data relevant to the questions raised above concerning the role of free amino acids in marine organisms. A portion of this work has appeared in abstract form (Stephens and Virkar, 1965).

Material and Methods

Ophiactis arenosa is a small brittle star which lives in close association with several sponges found on floating docks and on pilings. Animals were collected as required from Newport Bay south of Los Angeles. Masses of sponge were brought into the laboratory and kept in sea water. The brittle stars emerged on the surface of the sponge mass in about an hour and were placed in sea water in dish pans. Several hundred animals were kept in a single pan in an incubator at a temperature of 15–16° C. Observations were carried out at room temperature (about 21° C.). Individuals used in the observations reported were selected in the size range of 10 to 30 mg. wet weight except for the data concerning the relation between weight and rate of uptake of glycine.

These animals may be exposed to moderate salinity variations in their normal environment but presumably do not suffer rapid changes. However, they proved capable of surviving a direct change from full-strength sea water to 60% sea water. Acclimation to 60% sea water was necessary for survival at 50% sea water. One set of observations is based on the responses of organisms transferred abruptly to 60% sea water. Aside from this, animals were allowed to adapt by placing them one day at 90%, 80%, and 70% sea water successively. They were kept for two days at 60% and 50% sea water. Observations were undertaken after all animals had been acclimated in this fashion. Dilutions of sea water were prepared with distilled water. The salinity of the sea water stock was 33.08‰.

Water content was determined by weighing individuals after drying on filter paper and reweighing them after approximately 24 hours at 110° C. Amino acid determinations were carried out by measuring ninhydrin-positive material using extracts in cold 80% ethanol. We used a technique described by Clark (1964) and are indebted to her for earlier personal communication of the method. The procedure was calibrated periodically using glycine standards, and such standards were determined routinely with unknown samples. The ninhydrin-positive material

in the extracts is treated as free amino acid and concentrations expressed as millimoles amino acid per kg. body water.

Uptake of amino acids was measured by supplying randomly labelled glycine-C^{14} or *l*-isomers of the other amino acids employed. Concentrations greater than 10^{-6} moles per liter were obtained by adding unlabelled amino acid. Determinations of radioactivity were made using a thin-window gas flow detector system. Animals were exposed to sea water solutions of labelled amino acids for a predetermined time. Initial and final radioactivity in the sea water was determined. Each individual was extracted for 24 hours in 2.0 ml. of 80% ethanol. Five-tenths-ml. samples of this extract were evaporated on planchets and counted. Each individual was then ground in 2.0 ml. of distilled water and 0.5-ml. samples of the brei evaporated on planchets. All data presented have been corrected for background and sample thickness.

Care was taken to insure that the data collected in one particular set of observations would be internally comparable by preparing labelled solutions from a single stock to the same final concentration. Thus no corrections for small differences in ambient radioactivity were required.

Descending paper chromatograms were prepared using *n*-butanol-acetic acid-water (120:30:50) followed by phenol-water (80% by weight) as described by Smith (1960). One-dimension descending chromatograms were also prepared using *n*-butanol-acetic acid-water. Autoradiographs were prepared by exposing Kodak No-Screen x-ray film to the chromatograms for a seven-day period.

Results

When exposed to a solution of glycine-C^{14}, uptake of the radioactive label is rapid and approximately linear for at least 30 minutes. Under normal circumstances, the greater part of the radioactivity is in the alcohol-soluble fraction while only a small percentage of the total is found in the brei. At the end of a 30-minute exposure, the ratio of alcohol-soluble to alcohol-insoluble radioactivity is of the order of 30:1.

A one-dimensional chromatogram of the alcohol extract of *Ophiactis* shows several ninhydrin-positive spots. The most prominent have R_f values which agree with those of glycine, alanine, taurine, and threonine. In both one- and two-dimensional chromatograms, autoradiographs show radioactivity in the region identified as glycine. Hence, it appears that the great bulk of the radioactivity is still in the form in which it was supplied. This was also true of an alcohol extract prepared from animals which had been sacrificed 24 hours after a 30-minute exposure to labelled glycine.

A number of experiments were performed using a larger brittle star, *Ophionereis annulata*. The animals were induced to autotomize their arms, and uptake of glycine by the isolated arms was measured. With suitable corrections, it appears that this preparation is about as effective as the whole animal, at least for three or four hours. Consequently, it is likely that the gut is not involved in any extensive way in this uptake. This would agree with previous reports (Stephens, 1962, 1963, 1964) for other invertebrates, and very probably applies to *Ophiactis* as well.

Observations were undertaken to relate uptake of labelled glycine to the weight of the animals. When the log of uptake was plotted against the log of wet weight,

a regression line of slope 0.545 was calculated by the least squares method. Rather than correcting for weight based on the exponential relation this implies, weights of the animals employed for subsequent observations were kept as closely comparable as possible and within the range of 10 to 30 milligrams. Uptake is expressed as cpm./mg. for animals in this range.

Observations relating uptake to ambient concentration of amino acid were carried out using glycine, valine, alanine, and arginine. When the data were plotted as the reciprocal of concentration against the reciprocal of uptake, the straight line which was expected was not obtained. Uptake was systematically too high at high concentrations for all of the amino acids used. A more extended series of

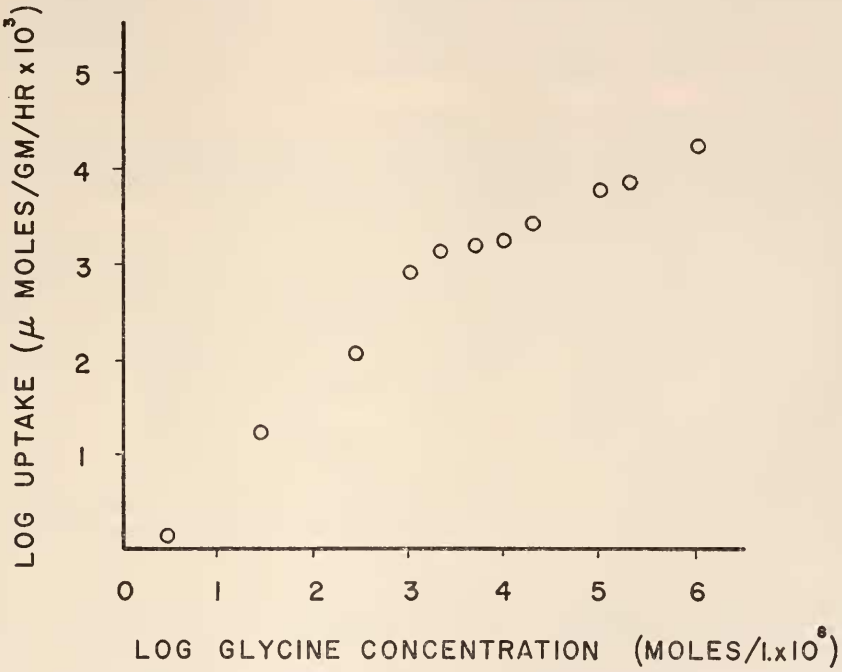

FIGURE 1. Rate of glycine uptake by *Ophiactis* as a function of ambient concentration of glycine. Each point represents the average value for ten or more individuals.

concentrations of glycine was used, covering the concentration range from 2.6×10^{-8} to 10^{-2} moles per liter. The data are presented in Figure 1. It will be noted that the accumulation system is not saturated at the highest concentration used. This differs from the relation reported for *Fungia, Clymenella, Nereis* sp., *Golfingia,* and a number of other invertebrates which show a definite maximum rate of accumulation (Stephens, 1962, 1963, 1964; Virkar, 1963, and unpublished observations). At the end of a 30-minute exposure to the lowest concentration employed (2.6×10^{-8} *M*), about 64 times as much labelled carbon per kilogram of water was found in the alcohol-solution fraction of *Ophiactis* as was present in the ambient solution. The lower limit in concentration was imposed by the specific activity

TABLE I

Average radioactivity in the alcohol-soluble and alcohol-insoluble fraction of Ophiactis at various times after a 30-minute exposure to glycine-C^{14} (U.L.). The data are presented as cpm./0.5 ml. extract divided by weight in mg. Standard deviations are included; n is 10 for all groups

Time (hours)	(cpm./mg.) alcohol-soluble	(cpm./mg.) alcohol-insoluble
0	117 ± 32	2.7 ± 0.7
0.5	108 ± 24	3.6 ± 0.9
1	106 ± 24	4.7 ± 1.2
2	111 ± 28	5.4 ± 0.9
4	106 ± 24	9.7 ± 2.3
6	86 ± 25	11.5 ± 2.7
24	45 ± 7	24.2 ± 4.4

of the labelled glycine and does not reflect a limitation of the physiological system involved. As is the case in forms previously examined, this accumulation system is essentially one way; no significant exchange of labelled material for unlabelled amino acid in the ambient medium was obtained.

When animals were exposed to glycine-C^{14} for 30 minutes and then allowed to remain in sea water for various periods subsequent to this exposure, there was a gradual increase in radioactivity in the alcohol-insoluble fraction of the animal. Table I lists the alcohol-soluble and alcohol-insoluble radioactivity at various times after a 30-minute exposure to labelled glycine. It is apparent that total radioactivity decreases with time although the alcohol-insoluble fraction increases in absolute level and not merely as a ratio.

At least a portion of the radioactivity which is lost from the system represents C^{14}-labelled carbon dioxide. Water in which brittle stars have been placed for 24 hours after an exposure to labelled glycine shows some radioactivity. This disappears on acidification and can be trapped on alkali in a Conway diffusion flask. Table II presents a balance sheet accounting for 92% of the radioactivity initially present in the system. A later experiment showed that CO_2 was lost to the atmosphere before acidifying. About three-quarters of the radioactive CO_2 was trapped on alkali before the sea water was acidified. The measurements presented in Table II are thus systematically low. The estimated correction for loss to the atmosphere (the parenthetical figures in the table) is probably too large since

TABLE II

Assimilation of glycine-C^{14} by Ophiactis during 24 hours following a 30-minute exposure. Radioactivity is expressed as counts per minute per milligram. The parenthetical figures include an estimate for $C^{14}O_2$ lost to the atmosphere based on separate measurements; n = 10 for all groups

Time	Alcohol extract	Brei	Medium	Total
0	573 ± 41	10.4 ± 1.9	—	583
24 hrs.	360 ± 67	150 ± 27	27 (114)	537 (624)

the free surface was not comparable in the two situations, but some additional CO_2 was certainly evolved.

A series of observations was undertaken to explore the effect of various inhibitors on accumulation of glycine by *Ophiactis* and its assimilation into alcohol-insoluble compounds. In each case, the animals were kept in solutions containing the inhibitor for one hour prior to exposure to labelled glycine in the presence of the inhibitor. Inhibitor concentrations of 10^{-3}, 10^{-4}, and 10^{-5} *M* were employed (except for N_2 where the animals were kept in nitrogen-saturated sea water). The data are summarized in Table III.

It is apparent that both accumulation and assimilation can be inhibited by a variety of agents. There is no reason to think that any of the inhibitors used has a specific effect on the system mediating accumulation. Both processes are sensitive to all of the compounds used. Assimilation seems to be reduced more than uptake in almost all cases. The inhibition noted probably reflects no more than

TABLE III

Percentage of inhibition of accumulation and assimilation of glycine by the agents listed at the concentrations indicated. Glycine concentration was approximately 10^{-6} moles per liter. All differences are significant at the 1% level

Concentration / Inhibitor	10^{-3}		10^{-4}		10^{-5}	
	Alcohol	Brei	Alcohol	Brei	Alcohol	Brei
KCN	33%	66%	—*	58%	—	39%
2,4-DNP	47%	83%	26%	—*	—*	—*
IAA	36%	74%	—	47%	—	45%
N_3^-	—	25%	—	—	—	—
N_2‡	43%	54%				

* Difference at the 5% level of significance.
‡ Nitrogen-saturated sea water.

the general dependence of the organism on oxidative metabolism. It may be noted that these animals do not survive for more than a few hours in nitrogen-saturated sea water. The responses of *Ophiactis* contrast with the insensitivity to various inhibitors reported for *Clymenella* and the latter's capacity to tolerate long periods without oxygen (Stephens, 1963).

The water content of animals acclimated to salinities between 100% sea water and 50% sea water was determined. Measurements were also made of the alcohol-soluble ninhydrin-positive material. These data are presented in Figure 2. The ninhydrin-positive material is treated as free amino acid. Ten amino acids were tentatively identified from chromatograms on the basis of their R_f values. In view of the reports of taurine in echinoderms (Kittredge *et al.*, 1962), the o-phthalaldehyde color reaction (Smith, 1960) was used to check for its presence in chromatograms. It was shown to be present by this criterion. However, the largest single constituent in the amino acid pool was glycine.

It is clear that decreasing salinity is correlated with a decrease in ninhydrin-positive material in alcohol extracts. Uptake of glycine and of valine was observed

at various salinities, and assimilation into alcohol-insoluble material also determined. The measurements of radioactivity were made on alcohol extracts and breis prepared after a 30-minute exposure to labelled material and a brief rinse in sea water. Figure 3 summarizes the results for a typical set of observations employing glycine. The initial increase in the rate of accumulation as slightly lower salinities are encountered by the organism is a constant feature of our observations of this kind. We also consistently find an increase in the percentage of labelled material

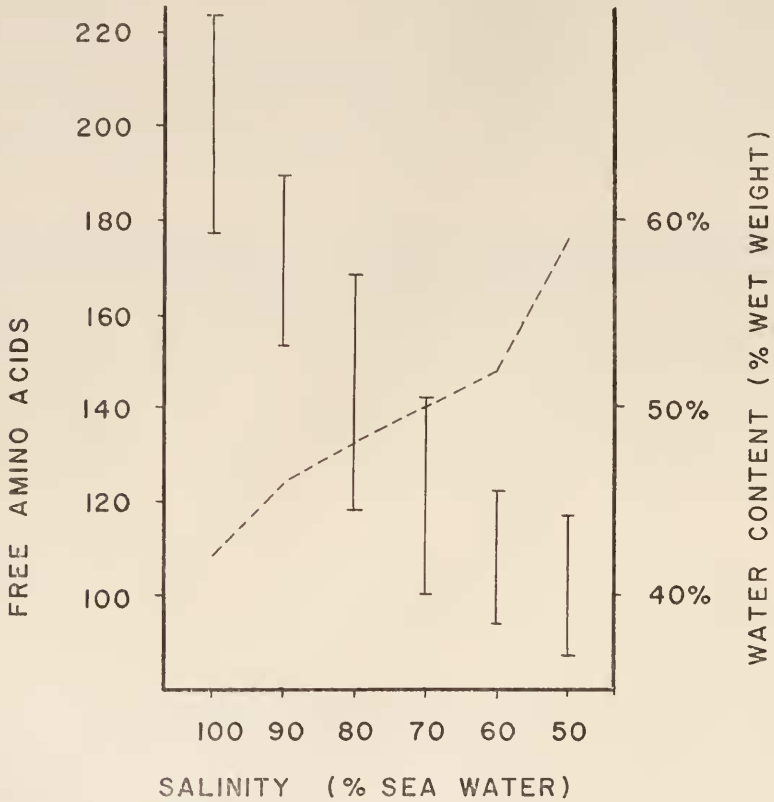

FIGURE 2. Total free amino acids (expressed as millimoles per kilogram body water) and water content of *Ophiactis* as a function of external salinity. Vertical bars represent standard deviations for amino acid values, dashed line the water content; n is 10 for amino acid determinations, 5 for water content.

accumulated which is assimilated into an alcohol-insoluble form. Observations with valine are similar. However, although the ratio of C^{14} assimilated increases with decreasing salinity, the absolute rate at which C^{14} appears in breis prepared after exposure at low salinities (50%, 60%) may decline. In any case, the specific stimulation of the assimilation of labelled carbon into the alcohol-insoluble fraction, concomitant with decreased uptake, contrasts with the effect of all inhibitors studied.

A set of observations was carried out to study the time course of the change in level of the free amino acid pool as well as the time course of the changes in accumulation and assimilation. Animals were transferred from 100% sea water to 60% sea water. Groups of ten animals were exposed to glycine-C^{14} and sacrificed at intervals for ten days following the transfer. The radioactivity of the

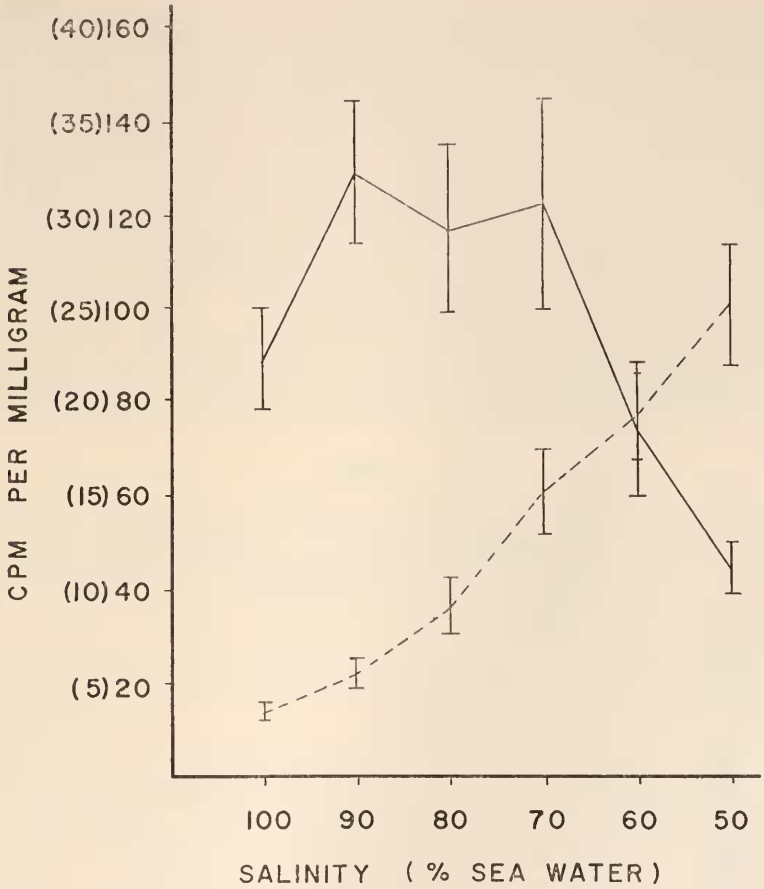

FIGURE 3. Radioactivity (expressed as counts per minute per milligram in 0.5 ml. extract or brei) recovered in the alcohol-soluble (solid curve) and alcohol- insoluble (dashed curve, parenthetical figures on ordinate) fractions of *Ophiactis* adapted to various salinities. The animals were exposed to glycine-C^{14} in their respective media for 30 minutes; n is 10 in all cases. Vertical bars represent standard deviations.

alcohol-soluble and the alcohol-insoluble fractions was determined for each animal. Measurements were made of the free amino acid pool in each group. A control group which was kept in 100% sea water under otherwise comparable conditions was sampled during the same period. Figure 4 presents the change in the free amino acid pool during the ten-day period for the two groups. Figure 5 presents

the percentage of the total radioactivity which was found in the alcohol-insoluble fraction of the animals. It will be noted that the response of the free amino acid pool is rather slow. This is also true with respect to the stimulation of incorporation of radioactivity into alcohol-insoluble compounds.

Discussion

Like almost all other marine invertebrates which have been examined, *Ophiactis* is capable of removing amino acids from extremely dilute solution in the surrounding sea water. The failure to obtain a definite maximum velocity of uptake contrasts with results which have been reported previously. The continued increase in rate which was observed over an ambient concentration range covering six orders of magnitude suggests a diffusion process. However, we will note later that the "free" glycine pool exceeds even the highest of the external concentrations employed by a factor of ten. It should be reemphasized that there is an overall accumulation of amino acids, that amino acids do not freely exchange with the medium, and that the concentration of amino acids in the normal habitat of these organisms must be very low compared to the intracellular pool.

Little is known about the feeding habits of this particular animal. It is also difficult to defend any very specific comments about the possible contribution that might be made by dissolved organic compounds. The close association of *Ophiactis*

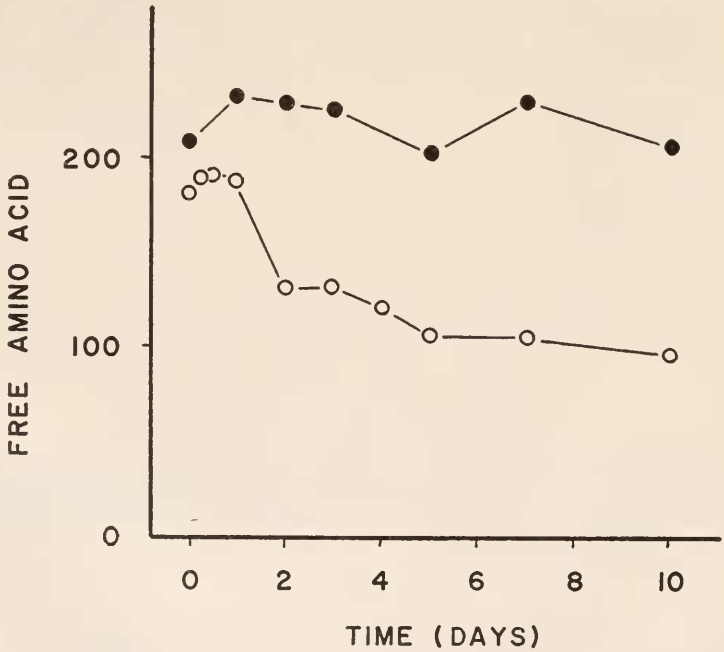

Figure 4. Time course of change in free amino acid concentration of *Ophiactis*. Concentration is expressed as millimoles per kilogram body water. Animals were transferred from 100% sea water to 60% sea water at zero time. Open circles, 60 % sea water; solid circles, controls in 100% sea water. Each point is average of ten animals.

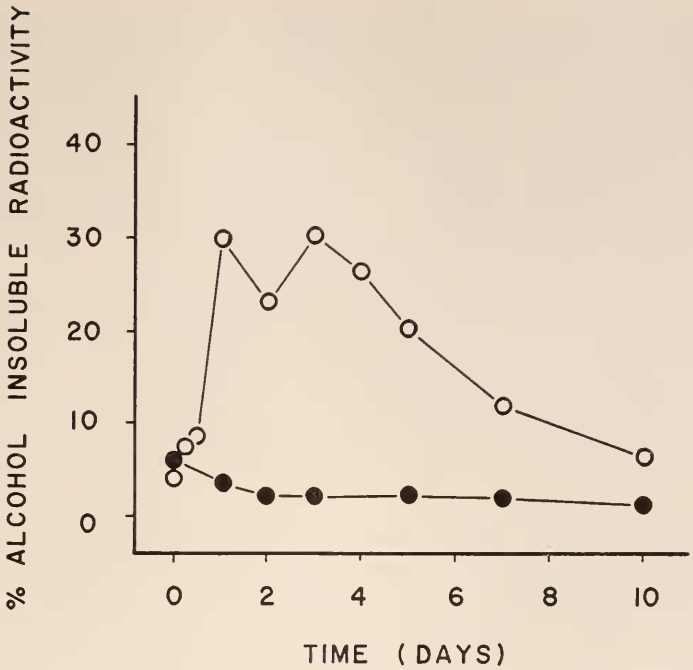

FIGURE 5. Radioactivity in the alcohol-insoluble fraction expressed as percentage of total radioactivity in *Ophiactis* exposed to glycine-C^{14} for 30 minutes at various periods of time following transfer to 60% sea water. In every case, ten animals each in 60% and 100% sea water were used. Open circles, 60% sea water; solid circles, 100% sea water.

with mussel beds and with sponges involves a microhabitat concerning which we have no chemical information whatever. Nonetheless, it is worth looking at the relation between the observed rate of amino acid accumulation and the metabolic needs of the animal.

Measurements of the oxygen consumption of animals in the size range of 10 to 30 milligrams wet weight, using a Gilson respirometer, gave approximately 0.147 ml. O_2/gm./hr. This is roughly equivalent to 147 micrograms of glycine/gm./hr. Our measurements indicate that the animals can obtain 64 micrograms/gm./hr. at an ambient concentration of 10 micromoles glycine per liter (0.75 mg./l.). This is roughly the concentration of glycine measured in the interstitial water of mud flats (Stephens, 1963) and in inshore water samples (Belser, 1959, 1963). This represents 43% of the organic material necessary to support the observed oxygen consumption. Since we have no information concerning conditions in the immediate environment of these brittle stars, we can only note that very modest ambient concentrations would permit this pathway to make a significant contribution to the energy needs of the organism.

The production of $C^{14}O_2$ indicates that the amino acids entering the organism are available for oxidation. Assimilation of labelled carbon into the alcohol-insoluble fraction implies that this material contributes to synthesis pathways. A rough estimate concerning the relative magnitude of the contribution of the

amino acid pool to energy metabolism can be made. Although complete quantitative information concerning the individual amino acids in alcohol extracts is not available, rough estimates were made by comparing chromatograms with controlled chromatograms of known amounts of glycine and taurine. An estimate of 0.1 mole of glycine per kilogram of body water is reasonable for normal salinities. At least 5% of the labelled glycine which is accumulated appears as carbon dioxide in our observations (Table II). This implies that this percentage of the pool has been oxidized. This figure may be higher if one assumes that the labelled carbon which disappears from the system in the observations summarized in Table I represents oxidized material. The figure would then rise as high as 40%. The glycine pool represents about 7.5 mg./g. body water or about 3 mg./gm. wet weight at normal salinities. Hence 0.15 to 1.2 mg. of glycine enters oxidation pathways. The daily requirement on the basis of O_2 consumption is about 3.5 mg. Hence glycine might account for approximately 4% to 34% of this requirement. Although the estimate is rough, it probably brackets the typical contribution of the glycine pool to energy metabolism and indicates that it is ancillary and not the primary energy source.

We can estimate major outputs which influence the size of the glycine pool in these animals. Energy metabolism drains 0.15 to 1.2 mg. of glycine per gram wet weight from the pool per day. Assimilation into alcohol-insoluble compounds removes about 12% of the pool per day (Table I) or about 0.36 mg. per gram wet weight. We can ask whether rates of uptake measured in these animals could contribute to maintaining the pool in the face of these deficits. Again, we must simply assume some reasonable ambient concentration failing direct information. If we accept the figure of 10 micromoles per liter suggested above, the input to the pool amounts to slightly more than 1.5 mg. glycine per gram wet weight per day. The fact that this figure balances the losses indicated so closely is of course gratuitous. However, one may suggest that the uptake of amino acids from the ambient medium is potentially capable of maintaining the size of the free amino acid pool and supplying the known drains on that pool. This suggestion rests on the assumption that the modest amounts of free amino acid stipulated occur in the specialized habitat of this organism.

In common with many other marine invertebrates, *Ophiactis* responds to a reduction of salinity by a decrease in the pool of alcohol-soluble ninhydrin-positive materials. If allowance is made for the increased water content of the organisms, the pool decreases to about 83% of its normal size in 70% sea water. Further apparent decreases at 60% and 50% sea water are produced almost entirely by the increase in water content of the organisms. It should be noted that the present data are not comparable to those reported by Virkar (1963, 1965) in which a large initial response of the free amino acid pool was noted. It was possible to distinguish between intracellular fluid and coelomic fluid in the case of *Golfingia*. Since no such distinction was made in *Ophiactis*, one cannot directly assess the effectiveness of the reduction of the free amino acid pool in the latter as an osmoregulatory response. However, there is no inconsistency in the two reports.

Our observations indicate that the effect of a small decrease in salinity is an increase in both the rate of accumulation and the rate of assimilation of amino acids. As salinity is reduced further, the rate of accumulation drops but assimilation

increases in rate. At 50% sea water, the rate of uptake of glycine is about half its value at normal salinities but the rate of assimilation has increased by a factor of seven. If lowering the salinity merely interfered with the energy metabolism of the organisms, we would expect a decrease in the rate of assimilation as was the case with all the metabolic inhibitors employed.

There are two major ways in which one might account for the increase in rate of assimilation at reduced salinities. They are not mutually exclusive. As the size of the pool decreases, there would be an apparent increase in the rate of assimilation in our experiments because of the increased specific activity of labelled glycine in the pool. This may be an element in the observed response. It does not seem to be a sufficient explanation. The total decrease in alcohol-soluble amino acids amounts to about 50% ; the apparent increase in rate of assimilation of glycine is about 700%. Although previous reports have indicated that particular amino acids such as glycine and alanine may be disproportionately involved in responses to salinity change, chromatography does not indicate any change in concentration of glycine of this magnitude in our animals. Hence, the specious increase in assimilation rate which would be produced by the decrease in size of the pool is probably not the most important effect reflected by our data.

The other possibility is that a decrease in salinity may produce an increase in the rate of incorporation of free amino acids into polypeptide and that this is the cause of the decrease in size of the pool. This seems reasonable. Incorporation of the free amino acids into an osmotically inactive pool would be the most economical way to reduce the pool. It it apparent from the magnitude of the increase in rate of assimilation that this could provide a rapid adjustment. At normal salinities about 3% to 5% of the pool is assimilated in a 30-minute period. In 50% sea water, about 36% of the pool is assimilated in the same time.

There is an apparent discrepancy between the time course of the change in the free amino acid concentration of *Ophiactis* (Fig. 4) and the time course of stimulation of incorporation of radioactivity into the alcohol-insoluble fraction of the animals (Fig. 5). The drop in the size of the pool follows this stimulation by as much as a day. A decrease in salinity must also produce an increase in the rate at which glycine is returned to the pool, so that it is more accurate to think of the salinity stress as stimulating both the exit from and the entry into the pool. The acclimation in turnover rate which is observed after several days in 60% sea water is accompanied by a maintained lower level of free amino acids. Presumably a new steady-state balance between inputs and outputs is reflected in the data. This slow change is reminiscent of the slow acclimation to increased salinity of the accumulation system in *Nereis limnicola* (Stephens, 1964). A detailed explanation of the decline in alcohol-insoluble radioactivity after prolonged exposure to reduced salinity awaits further work.

Since labelled carbon dioxide is produced, metabolism of the free amino acids cannot be limited to the shuttling back and forth from polypeptides to the pool which the autoradiographs might suggest. The specific activity of the glycine pool is very low despite considerable radioactivity in the alcohol-soluble fraction because of the remarkable concentration of the free amino acid pool. It is probably this which accounts for the failure to note intermediate compounds in oxidative pathways by autoradiography.

The pattern of regulation in *Ophiactis* which is suggested by these observations depends on regulation of the rate of synthesis and breakdown of polypeptide in response to a change in salinity. It is surprising to find that the initiation of these changes is as slow as it seems to be. The fact that 24 to 48 hours are required to produce a reduction in free amino acids in response to a sharp challenge suggests that the initial survival of the animal depends on its capacity to accommodate to drastic change. The great diversity of osmoregulatory mechanisms which have been described with regard to inorganic ion regulation make it very risky to attempt to generalize from the work we are reporting. We contemplate studies of other euryhaline animals to provide comparative data.

Summary

1. The brittle star *Ophiactis arenosa* shows uptake of C^{14}-labelled glycine, valine, alanine and arginine from dilute solution. The process is linear with time for at least 30 minutes.

2. The bulk of the radioactivity accumulated during a 30-minute exposure to glycine-C^{14} remains in alcohol-soluble form. Autoradiography reveals the radioactivity to be associated with glycine.

3. If the animals are allowed to remain in sea water following such an exposure, there is a gradual assimilation of the label into alcohol-insoluble compounds. Some radioactivity appears as $C^{14}O_2$, implying oxidation of the amino acid.

4. A double-reciprocal plot of concentration against rate of uptake does not give a straight line. Even at ambient concentrations as high as 10^{-2} *M*, the accumulation system apparently is not saturated.

5. Common metabolic inhibitors decrease the rate of both accumulation and assimilation.

6. The free amino acid pool of *Ophiactis* in 100% sea water is of the order of 200 m*M*/kg. body water. In animals subjected to reduced salinities, there is a decrease in the size of the pool corresponding to the degree of dilution of the medium.

7. One- and two-dimensional chromatograms of alcohol extracts of the animals show several ninhydrin-positive spots, of which glycine, alanine, threonine, and taurine are most prominent.

8. When animals maintained at reduced salinities are exposed to labelled glycine or valine, the response to modest decrease in salinity is a stimulation of uptake. As salinity is decreased further, there is a decrease in the rate of accumulation. In all cases, however, there is a marked increase in the rate of assimilation of the accumulated material into alcohol-insoluble compounds.

9. The response to reduced salinity, with respect to both the size of the free amino acid pool and the incorporation of the label into alcohol-insoluble fraction, is slow, occurring over a period of several days.

10. The significance of the results is discussed in terms of the energy relations of the animals, and the functions of the free amino acid pool.

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